

## SUBLETHAL EXPOSURE TO A NEUROTOXIC PESTICIDE AFFECTS ACTIVITY RHYTHMS AND PATTERNS OF FOUR SPIDER SPECIES

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**ABSTRACT.** Four species from three families of spiders were exposed to sublethal concentrations of the neurotoxic pesticide malathion: *Schizocosa ocreata* (Hentz 1844), *Rabidosia rabida* (Walckenaer 1837), *Frontinella communis* (Hentz 1850), and *Salticus scenicus* (Clerck 1757). Spider activity was recorded using a proprietary computer vision system equipped with artificial intelligence routines. Exposure to malathion changed the spiders' propensity to move, levels and patterns of activity, and distance moved. Dosed spiders increased their activity between 12 and 40%, depending on the species. Continuous recordings for  $\geq 24$  h revealed the peak activity for dosed *R. rabida* and *S. scenicus* was shifted  $\sim 1$  h earlier than controls. Spiders exposed to malathion also significantly increased the distance they moved per locomotory bout. This is consistent with the action of an organophosphate neurotoxin acting as an acetylcholinesterase inhibitor. Thus, exposure to sublethal doses of malathion appears to affect the neural basis for these spider's normal diel periodicities, time budgets, and patterns of locomotion, probably reducing their efficiency as agents of biological control.

**Keywords:** *Rabidosia*, *Salticus*, *Schizocosa*, *Frontinella*, malathion, behavior

Many insecticides kill arthropods by affecting the nervous or endocrine systems. Since an animal's behavior is governed by interactions among nerve and/or endocrine cells, it is not surprising that low and sublethal doses of pesticides influence behavior. Insecticides used to control insect pests can also affect spider populations either directly (through death) or indirectly (via changes in behavior or physiology). Given the likely importance of spiders in insect control (Mansour et al. 1980; Riechert 1998, 1999; Maloney et al. 2003), pesticide toxicity has been evaluated for these important predators. Where the susceptibility of several spider species to 30 pesticides was tested, toxicity ranged from no mortality (biological compounds, herbicides, fungicides), to medium mortality (pyrethrins, organophosphates, carbamates), and high toxicity cyclo-compounds (Mansour & Nentwig 1988).

Field trials investigating the impact of some toxins on spiders have shown varying responses. The application of broad-spectrum insecticides in apple orchards significantly de-

creased spider and harvestman populations (Epstein et al. 2000). Populations of lycosid, linyphiid, and other non-insect arthropod predators (harvestmen and centipedes) in fields sprayed with organophosphates (at standard application rates) versus water did not change their densities as sampled by pitfall trapping (Hodge & Vink 2000). Other tests and field surveys have suggested that many insecticides have little effect on spider population densities (Riechert & Lockley 1984; Hilburn & Jennings 1988; VanDenBerg et al. 1990) leading Integrated Pest Management (IPM) researchers to rate the risk of these toxins to beneficial arthropods as low (Higley & Wintersteen 1992). A recent review, however, cautions that some pesticides, even in concentrations below recommended field application rates, can result in high mortality (Maloney et al. 2003).

Sublethal doses of insecticides may affect the physiology and behavior of insects (Haynes 1988; Longley & Jepson 1996; Delpuech et al. 1998; Venkateswara et al. 2005) and arachnids (Chu et al. 1976, 1977; Samu & Vollrath 1992; Amalin et al. 2000; James &

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Price 2002; Tietjen 2006). Low doses of malathion (1,2-di(ethoxycarbonyl) ethyl O,O-dimethyl phosphorodithioate) increased walking speed of wasps, while other compounds caused continuous wing-fanning and/or flight (Haynes 1988). For arachnids, exposure to formamidine compounds affected the activity and dispersal of some mites and ticks (Hollingworth 1976).

Most research on sublethal doses of pesticides has concentrated on the economically important insects (Haynes 1988) with few studies assessing their effects on spider biology even though spiders are important generalist predators. More investigations have focused on measuring direct mortality to spiders (Amalin et al. 2000; Epstein et al. 2000; Van Erp et al. 2002) rather than observing behavioral changes resulting from pesticide exposure (Samu & Vollrath 1992; Shaw et al. 2006). Hodge & Vink (2000) indicated that although lycosid and linyphiid spiders may not be good bioindicator candidates, they should be investigated to elucidate physiological responses from sublethal exposure. Individuals of *Lycosa hiliaris* surviving a single exposure to diazinon in the field showed 87% inhibition of cholinesterase activity (Van Erp et al. 2002), making this enzyme in wolf spiders a possible biomarker for organophosphate contamination.

Preliminary observations in the laboratory suggested that spiders dosed with sublethal concentrations of malathion were more active than control animals (Tietjen unpubl. data). To further explore this observation, methods were developed to determine if altered spider activity is measurable and if the underlying mechanisms producing these changes in activity could be identified. Because different species of insects have varying responses to insecticides (Haynes 1988), several spider species from differing families and with different foraging strategies were tested. Results show that exposure to malathion changed the spiders' propensity to move, their levels and patterns of activity, and the distance moved during locomotor activity.

## METHODS

**Test animals.**—Data were collected for the salticid *Salticus scenicus* (Clerck 1757), the linyphiid *Frontinella communis* (Hentz 1850), and the lycosids *Schizocosa ocreata* (Hentz

1844) and *Rabidosia rabida* (Walckenaer 1837). All spiders (except *S. scenicus*) were collected from an old field on the Kentucky State University Research Farm (38.2° N, 84.9° W) that had been pesticide-free for  $\geq 10$  yr. *Salticus scenicus* were gathered from the surface of old grain bins adjacent to the field. Spiders were collected during the 1996–2000 field seasons.

All species (except *Frontinella*) were housed in 14-cm diam  $\times$  2.5-cm high plastic Petri dishes positioned upside-down so water could be delivered in the gap between the top and bottom of the dish. A 2-cm diam access hole was cut in the top of the dish to permit delivery of food items (the hole was corked when not in use). *Frontinella* were housed in 8.5-cm  $\times$  5-cm dishes and provided water through a moistened cotton wad in the container lid. Spiders were fed a natural diet of appropriately-sized insects (obtained via sweep-netting) on alternate days. Filter paper was the substratum for all species.

For all species (except *S. ocreata*), penultimate or antepenultimate spiders were captured and raised to adulthood in the laboratory, and only adult females were used to reduce variance due to gender. *Schizocosa ocreata* spiderlings tested were from the same egg sac when  $< 1$  wk old. Data were collected during their early communal behavior before dispersal of the spiderlings, commencing on day-three post-eclosion.

**Dosing procedures.**—Adult female spiders were exposed for 24 h to 10  $\mu$ l of  $10^{-5}$  malathion (1,2-di(ethoxycarbonyl) ethyl O,O-dimethyl phosphorodithioate) (w/w distilled water) applied to the center of a filter paper substrate covering the bottom of a standard glass Petri dish. Control animals were exposed to 10  $\mu$ l of distilled water under the identical protocol. The 24-h exposure was chosen based on preliminary tests of mortality and to allow potential variation in individual activity to be summed over a day, thus minimizing variations in pesticide exposure among animals. Spiders were not exposed to pesticides by spraying because of potential problems delivering a replicable dose to the animals. Spiders were tested 24 h after exposure to allow for potential recovery and to ensure that no animals died as a result of pesticide application. *Frontinella* females were returned to their webs after exposure to the pesticide.

*Schizocosa ocreata* spiderlings could not be



Table 1.—Mean distance ( $\pm$  SD) moved by four spider species. "Dosed" spiders were exposed to malathion, while controls were not. Treatment group sample sizes are indicated in parentheses. The length of recording time for each experiment also is indicated. Statistical analyses depicted are from a one-way ANOVA across spider species.

Species	Time	Distance moved		ANOVA
		Control spiders	Dosed spiders	
<i>Schizocosa ocreata</i> , Lycosidae	15 min	1.1 mm $\pm$ 1.97 (20)	1.3 mm $\pm$ 1.94 (27)	$F = 8.81$ , $P < 0.003$
<i>Frontinella communis</i> , Linyphiidae	24 h	1.1 cm $\pm$ 9.6 (20)	1.5 cm $\pm$ 10.08 (20)	$F = 84.66$ , $P < 0.0001$
<i>Rabidosa rabida</i> , Lycosidae	24 h	3.5 cm $\pm$ 8.14 (40)	5.8 cm $\pm$ 9.72 (40)	$F = 272.72$ , $P < 0.0001$
<i>Salticus scenicus</i> , Salticidae	7 da	1.5 cm $\pm$ 1.39 (22)	1.7 cm $\pm$ 1.22 (17)	$F = 4.79$ , $P < 0.02$

dosed using this technique because they produced a silk platform separating them from contact with the pesticide. Therefore, a vacuum-dosing technique modeled after Pluthero & Threlkeld (1980) was used. Spiderlings were introduced into a chamber in groups of five. The atmosphere was partially evacuated from the chamber and then the chamber was pierced with a syringe needle containing 10  $\mu$ l of the dosing compound ( $10^{-5}$  malathion or distilled water). The sudden change in atmospheric pressure caused the solution to enter the chamber and vaporize. Spiderlings were covered with a fine mist and the compound was forced into the booklungs. Spiderlings remained in the chamber for 1 min and were tested 24 h later. Adult spiders were not dosed using this technique because preliminary work indicated that some would become active and contact the condensate on the interior of the dosing chamber. Others would remain inactive and had little excess exposure to the condensate. These differential behavioral responses to vacuum and the sudden return to normal pressure would increase dosage variability for the adults compared to the filter paper technique.

An initial examination of dosed animals found they were not overtly adversely affected by pesticide exposure. The coordination of their locomotion, grooming, and other general behaviors appeared to be normal when compared to control animals. A light tap on the cages of dosed spiders elicited a startle response that was not seen for control animals, but otherwise the general responses of the two groups were similar. None of the dosed ani-

mals nor the control animals died during the experiment (except a few *S. scenicus* during the long-term observations; see below).

**Recording spider activities.**—Spider activity was recorded using a computer-controlled digital camera (EDC 1000, Electrim Corporation, Princeton, NJ, USA). Methods were similar to those described by Tietjen (1980, 1981, 1982). During recording, spiders (except *Frontinella*) were housed in plastic Petri dish arenas with a filter paper substratum. The diameter of the arena was selected relative to the animal size (5 cm for *S. ocreata* spiderlings, 8.5 cm for *F. communis*, and *S. scenicus*, and 13.5 cm for *R. rabida*). Female *F. communis* were observed while on their own webs built in their container 2 wk before dosing. The camera was suspended from the ceiling by a tripod head that allowed the apparatus to focus on spiders of varying size. For each run a cm ruler was placed in the field of view to permit metric conversion.

**Spider recordings with single *S. ocreata*, *F. communis*, & *R. rabida*.**—One version of the camera software permitted the logging of a single spider at a time. This was used to record the activity of *S. ocreata* spiderlings and *F. communis* and *R. rabida* females. Recording intervals were set at one frame per 10 s (except for *F. communis*, which recorded at one frame per min). For each interval, the current time and X/Y coordinates of the spider were logged to a file. These coordinates were serially accumulated to enable frequency and distance measurements over time. For *S. ocreata*, movements were recorded between 20:00 and 21:30 (10-s intervals, Table 1) in

an effort to minimize circadian effects. Illumination for this species was provided by a 40 W incandescent lamp positioned at a 30° angle to the recording arena, approximately 2 m away from the test animals, and was not varied over the recording period for either species. Recordings of *F. communis* and *R. rabida* females ran for 24 h (1-min intervals, Table 1), and ambient illumination was provided by a skylight. Data collection alternated between recording dosed and control spiders. New arenas were used for each run.

**Long-term recordings with multiple *S. scenicus*.**—Improvements to the software allowed simultaneous recording of up to 10 animals individually-housed in Petri dishes for up to 7 d. For these observations, *S. scenicus* were tested in groups of 5 dosed and 5 control animals. The computer was programmed to continuously sample and adjust the camera's exposure, allowing automated recording of spiders under natural photoperiod provided by a skylight in the recording room. The system adjusted to passing clouds, bright sunlight, and inclement weather. During the evening hours infrared LEDs illuminated the recording area since the camera was sensitive to these wavelengths while spiders are not (Crain 1949; DeVoe et al. 1969). The temperature and humidity in the recording room tracked outdoor conditions. The arenas were surrounded with black paper rings to visually isolate the test animals. In addition, arenas were set in a sand basin placed on a granite base supported by partially-evacuated tennis balls. This apparatus dampened vibrations caused either by animal activities in adjacent arenas or human activities in a nearby room. Vibrations also were minimized by the first-floor location of the recording room and a concrete-slab floor separated from the rest of the building structure. (Vibration-reduction techniques were not employed when recording the activities of *S. ocreata*, *R. rabida*, and *F. communis*. This is not considered to be problematic for these species since their activities were recorded individually and not in groups of ten.) Sampling rates for groups of *S. scenicus* were one image/10 s. During the recording period, spiders were unattended to avoid external stimuli.

*Salticus scenicus* was chosen for the long-term observations since they were available as adults from late spring to early fall, whereas

*R. rabida* were available for collection for 3 wk and would survive as adults in the lab for only ~ 6 wk. Therefore, testing 80 *Rabidosa* for a week in groups of 10 would not have been possible. *Salticus scenicus* were undisturbed for each 7-da recording period. Water was supplied continuously to the edges of the arenas by a wicking system. Prey were not introduced while recording because previous observations indicated that dosed animals often did not attempt prey-capture, and the artificial intelligence routines of the machine-vision program were not sufficiently robust to reliably differentiate between spiders and prey located in the same container. Thus, feeding during the recording period would add an uncontrollable variable. Initiation of the experimental runs was timed with the feeding schedule so *S. scenicus* received food the day before the test. Of the 25 spiders in each treatment, one control and two of the dosed animals died during the long-term recordings. Two control and six of the dosed animals were removed from analysis because they appeared to suffer from dehydration caused by failure of the water-delivery system.

**Analyses.**—The activity levels, expressed as distance-moved, were analyzed using conventional statistics (Stata, Stata Corporation, College Station, TX, USA). For all but *S. ocreata*, the time that individuals moved were also analyzed with circular statistics (Batshelet 1965). In addition, *R. rabida* and *S. scenicus* data were teased-apart through a Fourier analysis (Tietjen 1982) to inspect frequency and duration information. Because the data were collected at 10 s intervals, over 2.3 million data points were generated from the long-term observations of *S. scenicus*, making data sets too large for the available commercial statistical packages. Therefore, data were pooled into 1-min intervals, reducing the data set to less than 400,000 points. Those one-minute intervals having no movement also were discarded, further reducing the data set to 43,000 points without affecting the analyses, and allowing the use of standard statistics packages. Although the original 10 s interval data sets for spiders (except *S. scenicus*) were considerably smaller, only the *F. communis* data could have been directly analyzed. Therefore, all 24-h data (*S. ocreata*, *F. communis*, *R. rabida*) were analyzed at a 1-min resolution.

Voucher specimens of spider species used



Table 2.—Circular statistical analyses for control and “dosed” (= exposed to malathion) spiders (*Fron-tinella pyramitella*, Linyphiidae; *Rabidosa rabida*, Lycosidae; *Salticus scenicus*, Salticidae). The mean vector ( $\mu$ ) is the mean peak activity “strength” expressed as the length of  $\mu$  ( $r$ ) and the circular standard deviation. Significance in the “Raleigh Test” column indicates that the spiders had a preferred time for peak activity. Significance for Watson’s F-test indicates that control and dosed spiders had a shift in the time of peak activity. Sample sizes are in parentheses.

Species and treatment	Mean vector ( $\mu$ )	Length of $\mu$ ( $r$ )	Circular standard deviation	Raleigh test of uniformity	Watson’s F-test for two circular means
<i>R. rabida</i> control (40)	08:16	0.232	94.80°	$P < 0.0001$	$F = 84.91, P < 0.0001$
<i>R. rabida</i> dosed (40)	07:25	0.254	97.99°	$P < 0.0001$	
<i>S. scenicus</i> control (22)	14:02	0.470	70.31°	$P < 0.0001$	$F = 462.92, P < 0.0001$
<i>S. scenicus</i> dosed (17)	12:45	0.654	52.74°	$P < 0.0001$	
<i>F. communis</i> control (20)	14:46	0.01	171.00°	$P > 0.05$	not applicable
<i>F. pyramitella</i> dosed (20)	07:08	0.08	129.75°	$P > 0.01$	

here are deposited in the arthropod collection of Miami University, Oxford, Ohio.

## RESULTS

### Distance moved and circadian rhythms.—

The distances moved per time period for all spider species significantly increased when dosed with malathion (Table 1). A circular analysis of the daily time data for *R. rabida* and *S. scenicus* indicated that their activity over 24-h periods was not uniform (Raleigh Test,  $P < 0.0001$ ; Table 2). In addition, there was a change in the mean peak activity time for these two species. Control *R. rabida* females had a peak activity at 08:16 while the peak activity for dosed animals was at 07:25, a progression of nearly 1 h (Fig. 1). Dosed *S. scenicus* females also shifted their peak activity to over an hour earlier in the day from 14:02 for the controls to 12:45 for the dosed spiders (Fig. 1). These activity shifts were significant for both species (Watson’s F-Test,  $P < 0.0001$ ; Table 2). Dosed female *S. scenicus* also showed several bursts of increased activity during evening hours between 22:00 and 05:00, while spurious activity was not apparent for *R. rabida* (Fig. 1).

Female *F. communis* movements also were analyzed using circular statistics. Control *F. communis* had a non-significant activity peak at 14:46 (Watson’s F-Test,  $P > 0.05$ ; Table 2). Dosed *F. communis* had a significant peak activity at 07:08 (Watson’s F-Test,  $P < 0.01$ ; Table 2). Because only one of the treatments had a significant activity peak, Watson’s F-Test for two circular means was not performed. The large circular standard deviation

(almost 180 degrees short of a full circle for the control group) may be an artifact caused by constant lighting conditions during the recording period. It is important to note, however, that this does not affect the analyses related to distance-moved since those data are independent of and not coupled to timing of activity bouts. Since both control and experimental spiders were tested under constant-light conditions, the differences in distance-moved can only be explained by the experimental protocol.

**Patterns of activity.**—Fourier analyses were performed on data recorded from *R. rabida* and *S. scenicus* to investigate the small-scale patterning of activity that summate to produce a circadian rhythm (Table 3; Figs. 2, 3). The power spectra for *R. rabida* indicate that dosed spiders showed higher frequencies at higher power compared to control animals (Table 3). Spectral components were generally more well-defined for dosed animals, and there were changes in the low-frequency portions of the spectra (Fig. 2). The shift by dosed animals to shorter time intervals with higher power reflects the earlier activity peaks observed in dosed *R. rabida* (Fig. 1).

An examination of the power spectra for *S. scenicus*, on the other hand, indicates a longer periodicity and lower power for dosed animals (Table 3). Also note that the power for each time period for dosed animals is less than half that seen in the controls (Fig. 3), which is a greater difference than observed for *R. rabida*. In addition, the power spectra for *S. scenicus* have a lower noise component when com-

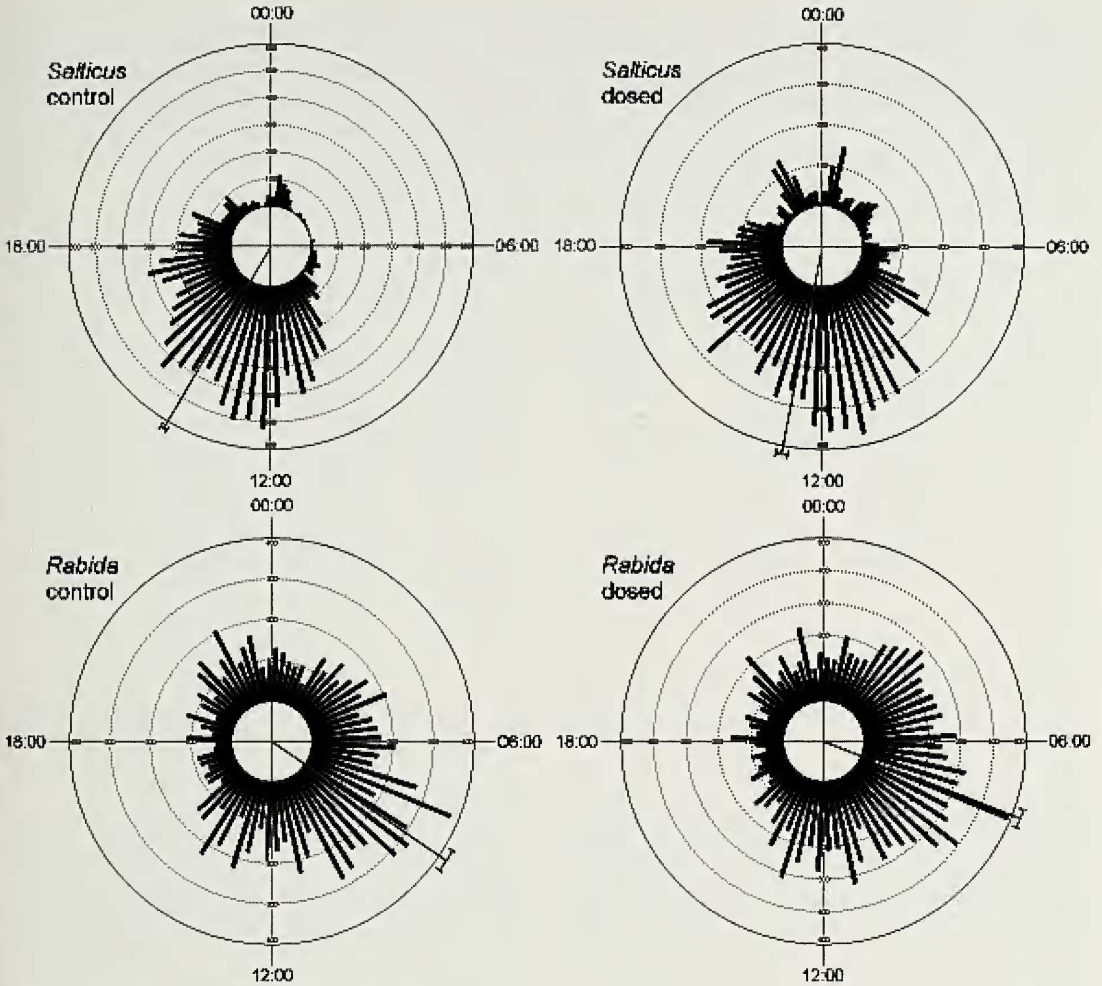


Figure 1.—Circadian responses of two spider species to malathion. Circular graphs depict a 24-h period to show changes in the circadian activity of control *Salticus scenicus* (Salticidae) and *Rabidosa rabida* (Lycosidae) compared to animals dosed with malathion. The indicated variations of the mean vectors are the 95% confidence limits. Sample sizes: *R. rabida* control (c) and dosed (d) = 40; *S. scenicus* c = 22, d = 17.

pared to that of *R. rabida* (compare Figs. 2, 3). Interestingly, many of the spectral components over most of the spectral range for dosed and control animals align with one another (Figs. 2, 3). Thus, a different mechanism must be responsible for the observed shifts in mean activity rhythms of *S. scenicus* versus *R. rabida*, suggesting different behavioral/physiological mechanisms controlling circadian rhythms in these two species.

An independent measure of the time-pattern elucidated by the Fourier analysis can be obtained by comparing the number of bouts of movement among the species and treatments. The number of movement-bouts for control *R.*

*rabida* was 11,304 while dosed animals significantly decreased their movement-bouts to 10,721 ( $\chi^2 = 15.43$ ,  $df = 1$ ,  $P > 0.001$ ). Thus, since dosed *R. rabida* moved longer distances than control spiders (Table 1), they must have a propensity to move farther once a movement-bout begins. Coupled with the higher frequency and power spectra illustrated from the Fourier analysis, these results explain the greater distance moved by dosed *R. rabida* compared to controls (Table 1).

The number of movement-bouts for dosed *S. scenicus* (28,957.18; adjusted) showed a significant increase of 29.4% compared to control animals (20,772;  $\chi^2 = 1347.24$ ,  $df =$



Table 3.—Power spectra of activity for control and “dosed” (= exposed to malathion) spiders *Rabidosa rabida* (Lycosidae) and *Salticus scenicus* (Salticidae). The ten strongest spectral components and their frequencies (expressed as time) are presented. Data are sorted in descending order based on power. Dosed *R. rabida* had higher frequencies at higher power compared to control animals, indicating earlier activity peaks for those spiders exposed to malathion. Dosed *S. scenicus* had longer periodicity and lower power than controls, showing dosed spiders had a shift of activity rhythm similar to *R. rabida*, but with a different pattern of activity from that of *R. rabida*.

<i>Rabidosa rabida</i>				<i>Salticus scenicus</i>			
Control		Dosed		Control		Dosed	
Time	Power	Time	Power	Time	Power	Time	Power
00:00:16	28,473.08	00:00:50	37,775.29	00:00:16	937,233.87	00:00:16	350,529.26
00:00:50	19,021.45	00:00:16	25,704.70	00:08:11	76,580.08	00:08:11	21,683.22
00:01:24	6,756.55	00:01:24	9,986.70	00:17:14	38,015.42	00:01:24	14,351.12
18:30:14	5,642.32	08:01:47	8,359.91	00:35:19	33,560.74	01:11:29	13,916.73
07:05:33	5,575.28	06:56:13	7,755.35	00:03:40	32,688.73	02:23:49	11,719.87
04:48:30	5,457.57	00:16:40	7,566.96	01:11:29	26,941.13	04:48:30	11,612.50
13:45:24	5,110.31	22:34:56	6,913.85	02:23:49	25,173.79	00:35:19	11,025.64
05:54:03	4,838.85	19:06:24	6,885.65	04:48:30	24,732.63	00:17:10	10,440.23
07:51:37	4,638.15	04:58:40	6,463.89	00:05:56	13,925.17	00:03:40	5,131.22
08:35:41	4,564.55	02:43:02	6,415.13	00:07:37	13,127.38	00:53:24	3,729.32

1,  $P < 0.0001$ ). Since the mean distance-moved increased only slightly from 1.5 cm for control animals to 1.7 cm for the dosed spiders (Table 1), the distance covered during a movement-bout must, on average, be shorter for dosed than control spiders, otherwise a greater disparity in the average distance moved would be expected. Since the data

were pooled into 1-min intervals, the more frequent short-distance movements would sum within a minute and could only be inferred through the above analyses.

The pronounced decrease of the power spectrum strength for dosed *S. scenicus*, coupled with the greater number of movement-bouts, implies that high-frequency compo-

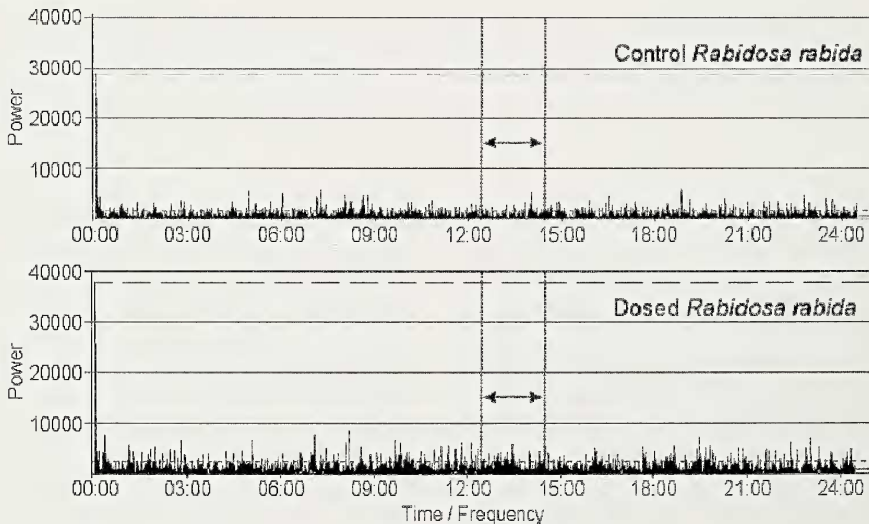


Figure 2.—Power spectra of activity by the spider *Rabidosa rabida* (Lycosidae). Spiders dosed with malathion exhibited a generally stronger spectrum than controls. The most significant spectral changes are with frequencies in the time period highlighted by the vertical lines. Abscissa represents time; ordinate depicts spectral power. Sample sizes: control and dosed = 40.

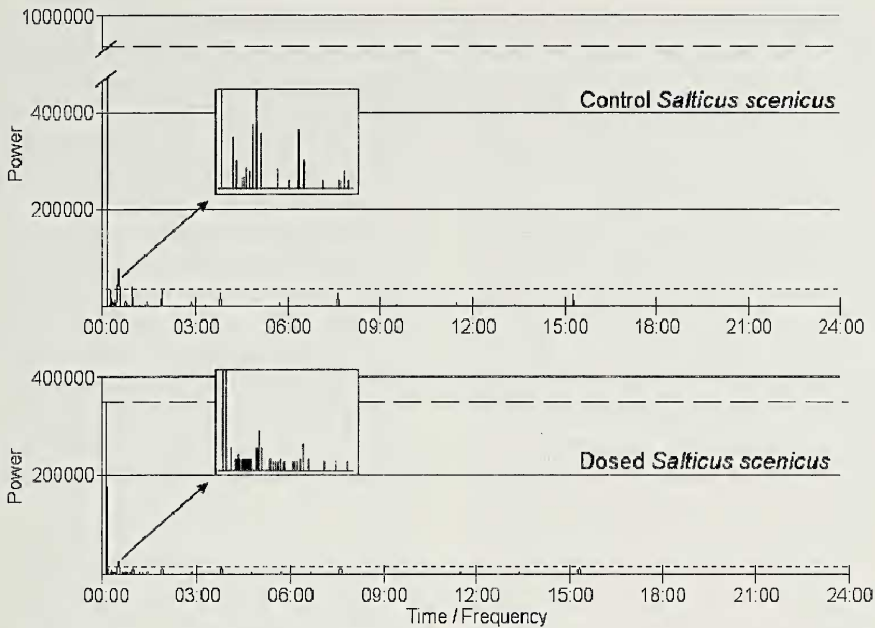


Figure 3.—Power spectra of activity for the spider *Salticus scenicus* (Salticidae). Spiders dosed with malathion showed a longer periodicity and lower power. Note that many of the spectral peaks align over time for the two treatments. Insets are enlargements of the spectra for the first hour. Abscissa represents time; ordinate depicts spectral power. Sample sizes: control = 22, dosed = 17.

nents of low power should be seen in the power spectrum. Indeed, close examination of the spectra reveals a large number of short-term frequencies of low power for dosed spiders not seen in the controls (compare insets in Fig. 3). These components reflect both the progression of more than an hour of the mean vector from circular analysis (Fig. 1) and the increase in movement-bouts by *S. scenicus*.

#### DISCUSSION

Sublethal exposure of these four spider species to malathion affected changes in the amount, duration, and patterns of their activities. Although there were no overt crippling effects to the spiders from this neurotoxin, the observed alterations of their behavior could have significant consequences to their ultimate survival. Furthermore, the spiders' efficiencies as predators could be impaired, decreasing their value as agents of biological control.

**Alterations of circadian rhythms.**—Changes in a spider's total and peak activity are likely to affect the time budget of the dosed animals (Cloudsley-Thompson 1960). All spider species tested here that could be fully analyzed using circular statistics became

active earlier in the day, possibly forcing them to forage when competitors and/or predators are present, or causing them to be active when their normal prey are unavailable. For example, field data suggest that *S. scenicus*' time budget is adjusted so their peak activity coincides to that of their primary prey on the surface of grain bins, the Angoumois grain moth, *Sitotroga cerealella* (Olivier 1789). In this specialized habitat, grain moths exit bins through the top vents between 13:00 and 14:30, probably in response to the mid-afternoon heat. Timed observations throughout the day indicated that during peak hours, 47% of grain moths on the bin surface were being fed upon by *S. scenicus* (1243 grain moths were scored), with only eight of the moths attacked by other spider species (Tietjen unpubl. data). In the present study, the daily period of greatest activity for control *S. scenicus* brackets the greatest availability of their primary prey at this location (Fig. 1). The shift of over an hour by dosed spiders could desynchronize them with the peak activity of their prey, reducing their predatory efficiency. Furthermore, the spurious activity bursts between 22:00 and 05:00 observed in *S. scenicus* could put them



at risk since these spiders rely heavily on vision for prey-detection and predator-avoidance.

Differences among the taxa tested were readily apparent, thus the effects of malathion on one spider species does not have the same effect on all species. Both *S. scenicus* and *R. rabida* showed an analogous mean daily activity change, but Fourier analyses indicated that different behavioral/physiological mechanisms must be responsible for the observed shifts in mean activity rhythms of *S. scenicus* versus *R. rabida*. Lycosids and salticids have differing levels of acetylcholinesterase activity in their protocerebrum (Meyer & Idel 1977). Perhaps the influence of malathion differentially influences neural integration in *S. scenicus* and *R. rabida*. The lack of spurious activity on the part of *R. rabida* compared to *S. scenicus* may be due to the lycosid's higher general activity throughout the day as evidenced by the smaller mean vector between treatments ( $r$  in Table 2; Fig. 1).

The circular analyses of *F. communis* showed a significant mean vector for dosed animals but not for the controls. Although the constant lighting conditions may have contributed to these effects, the mean activity peak for dosed animals suggests that exposure to malathion may affect the interactions between light receptors and the circadian clock. For example, the visual system may override the internal clock for control animals while dosed *F. communis* were not as strongly affected by the constant lighting conditions because of visual aberrations or a change in the coupling between photoreceptors and their base-line circadian rhythms affected by the inhibition of acetylcholinesterase.

**Altered locomotor behaviors.**—The propensity for dosed *S. scenicus* to move more frequently but for shorter distances may be due to visual distortions experienced by these spiders. While exploring the effects of malathion on prey-capture by *S. scenicus*, abnormalities in visual processing were discovered (Tietjen, unpub. data). Visual errors may cause dosed animals to move at a different rate or pattern. The protocerebral ganglia of salticids and lycosids show very high activities of acetylcholinesterase (Meyer & Idel 1977), which a center of vision for these animals (Meyer 1991). Since malathion is an acetylcholinesterase inhibitor, this pesticide

could impair visual processing and subsequent motor activities based on visual sensory input. If the time budget of *S. scenicus* includes a certain level of locomotor behavior, the short-duration spectra (depicted in the inset of Fig. 3) may represent a compensatory mechanism by the spiders to maintain a set amount of activity despite problems with visual processing or other integration systems. Apparently, in the presence of malathion, the underlying decision-making process over-compensates, resulting in a greater distance covered and a precession of the mean time of activity.

Pesticide exposure could cause different behavioral abnormalities at different life stages. Spiderlings, for example, may increase their general activity while adults may show a decrease. The increased activity of *S. ocreata* spiderlings after malathion contact could disrupt the duration of maternal care during a very vulnerable time in their lives. These spiderlings are carried on their mother's dorsal abdomen and cephalothorax for up to 10 days (Rovner et al. 1973). This affords them some protection and access to resources such as water and food while gathering strength. By dispersing from the mother too soon, dosed spiderlings would have increased exposure to predation, desiccation, and starvation, severely decreasing the probability of their survival.

**General and specific effects.**—The current analyses indicate that sublethal doses of malathion have generalized effects on behavior (such as increased activity) and also have specific effects on behavioral components, including the propensity to move, the distance a spider moves during an activity bout, and the small-scale patterning of locomotor behavior. Evidence of high acetylcholinesterase activity in the leg ganglia and connective ring systems of the ventral nerve cord in spiders (Meyer & Pospiech 1977; Meyer 1991) attests to the importance of proper acetylcholine regulation to locomotor coordination and control. Restricted inhibition of the stimulatory action from acetylcholine by malathion would lead to overactivity in these neural tracts central to locomotor activity.

Although one might expect that general behavioral alterations would be common, specific effects on spider behaviors by pesticides have been seen in other contexts. As an example, dosed *R. rabida* males exhibit completely normal courtship behavior in response

to female pheromone or in the presence of females (Tietjen 2006). However, when females indicated a willingness to mate, most dosed males were unable to shift from courtship behavior to copulation (40 control males mated, 2 of 40 dosed males; Tietjen 2006). In fact, all males that could not switch to mating behavior were killed by the normal females (Tietjen 2006). Cholinesterase activity was inhibited for laboratory male *Lycosa hiliaris* (Van Erp et al. 2002). Considering the neural integration and sensory feedback necessary to perform complex motor patterns (Milde & Seyfarth 1988; Gronenberg 1990), any impairment of neural transmission or control could significantly affect sexually-oriented behaviors.

The possibility that various neuropesticides could be used to elucidate the fine structure of behavior deserves future investigation. If different classes of pesticides affect different receptors and/or portions of the nervous system (Meyer & Idel 1977; Meyer 1991), it might be possible to use these chemicals as probes to selectively disable portions of a behavioral sequence and gain a greater understanding of the normal behavior of an animal.

Pest management researchers should carefully consider potential sublethal effects on the behavior, physiology, and reproduction of non-target species when planning an IPM scheme. This study suggests that malathion exposure may undermine the efficacy of spiders as biocontrol agents even if surveys find field population densities are not affected. Although live spiders are found to still occupy the treated areas, this study shows that these animals are probably behaviorally impaired. Studies should be performed on a wide variety of spiders from different families, life stages, and varying foraging strategies. There is the possibility that feeding on exposed prey may compromise the behavioral repertoire of otherwise unexposed spiders. In addition, data from the present experiments indicate that different species may have variable underlying responses to the same pesticide, further complicating assumptions in pest management when considering the biological component of IPM.

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